Structure and Mechanism of the DNA Polymerase Processivity Clamp Loader

D. Jeruzalmi, M. O'Donnell, and J. Kuriyan

The Rockefeller University, New York, NY

ABSTRACT:
The replication of a genome is a fundamental biological process that every organism must perform in order to transmit its genetic makeup to its progeny. We have been studying the molecular machines that are used to rapidly and accurately replicate the DNA genome of bacteria. In addition to the polymerase (the actual copying machine), organisms have a set of assemblies that enable the polymerase to perform its jobs more rapidly. One of these assemblies is the processivity clamp, which is a ring shaped protein that encircles and slides along DNA and physically tethers the polymerase to the DNA that is being replicated. The main problem that we have been studying is how does a ring-shaped sliding clamp become assembled onto DNA, which is a long polymer. Organisms have solved this problem by using a clamp loader machine that physically opens the ring and loads it onto DNA. We have determined the structure of an intact and functional clamp loader machine. This structure, along with a second structure of the clamp, caught in the act of being opened, has allowed us to construct a model of the clamp loading reaction.

Chromosomal replicases from prokaryotes to eukaryotes are organized around three functional components, a DNA polymerase, a ring-shaped processivity clamp, and a clamp-loading machine. DNA polymerases catalyze the addition of nucleotides to primed template DNA and, in isolation, are poorly processive enzymes (4,10,13). Presence of the processivity clamp, dramatically increase processivity. Processivity clamps are ring shaped proteins composed of 2 (eubacteria) or 3 (phage, archae, eukaryote) subunits (5). The clamps confer processivity by encircling DNA and physically tethering the polymerase subunit to the DNA as it tracks along the template (11). Assembly of sliding clamps on DNA is performed by the clamp loader assembly in a reaction fueled by ATP (12). In bacteria, the clamp loader is composed of 7 unique subunits (γ, δ, δ, χ, ψ) and the β_2 dimer is the processivity clamp. The clamp loader is an AAA+ ATPase and is the bacterial homologue of the eukaryotic replication factor C (RFC) (7, 8). We have determined the structure of the bacterial clamp loader $\gamma_3\delta\delta$' and that of the δ wrench in complex with a clamp subunit (β:δ). These structures were solved using data measured at the National Synchrotron Light Source (X-25) at the Brookhaven National Laboratory, at the Structural Biology Center at the Advanced Photon Source (ID-19), the Stanford Synchrotron Radiation Laboratory (9.2), and the Advanced Light Source (5.02) at the Lawrence Berkeley Laboratory.

The crystal structure of the β:δ assembly reveals that the δ subunit binds to the C-terminal side of the processivity clamp at a location near to, but not at the dimer interface (Figure 1) (3). We used a mutated (I272A, L273A) form of β (β_1), which exists as a stable monomer in solution and which binds to the wrench subunit (δ) with a 50 fold higher affinity than wild type (9). The δ subunit, which adopts the same fold as the other clamp loader subunits, places its N-terminal domain (β-interacting element) into a binding site composed of two domains (2 & 3) on β_1. Two highly conserved hydrophobic δ residues (F73, L74) are wedged into a hydrophobic pocket on the clamp. Binding of the δ subunit requires a conformational change in β that renders the clamp interface incapable of closing. With respect to its structure in the dimeric clamp, β from the β:δ complex adopts a conformation of reduced curvature. This observation along with molecular dynamics simulations suggest a spring-loaded mechanism in which the β ring opens spontaneously once a dimer interface is perturbed by the β wrench.

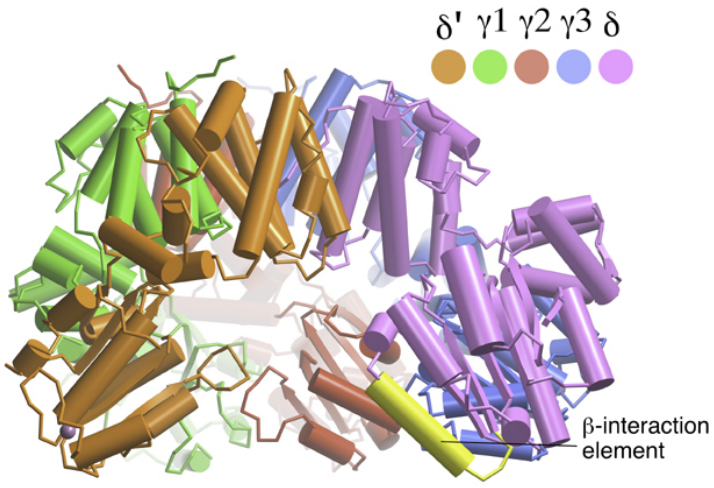

Figure 1. *Structure of the* β:δ *complex.*

Binding of the β subunit requires a substantial conformational change in the β clamp that renders it incapable of forming a closed ring. Clamp opening by the δ wrench exploits a peculiar structural feature at the β dimer interface. One element of the subunit interface of the dimeric clamp consists of the interfacial helix (α1"), whose last three residues (A271, I272 and L273) are distorted from ideal geometry. This distortion allows the side chains of residues I272 and L273 to fit into hydrophobic pockets on the surface of Domain I of the second β monomer. In the β:δ assembly, helix α1" is essentially undistorted, leaving it in a conformation that is incapable of forming a well-packed dimer interface with the second member of the dimer. Distortion of helix α1" is directly coupled to the binding of δ to β through a 5 residue loop in β (residues 274 to 278) that immediately follows it and which interacts closely with δ in the β:δ complex. The structure of this loop in β_2 is well defined due to the presence of a β turn between residues 275 and 278, but its conformation would prevent the binding of δ.

In comparison to β_2, β_1 of the β:δ complex is systematically distorted. Superposition of the β_1 onto domain 2 of β_2 reveals a strikingly relaxed structure. Domain 1 is rotated by 12° and domain 3 by 5° away from the central cavity. We have chosen to explain the observation that the largest deviation from the dimeric clamp occurs distant from the β binding site by proposing that the closed β dimer is under spring tension. The wrench subunit might therefore open the clamp by disrupting one of the dimer interfaces; clamp opening would occur spontaneously through release of the spring tension. A series of molecular dynamics calculations recapitulate the structure of the relaxed β monomer starting from the strained dimeric clamp.

The conformational change seen in the β subunit allows a plausible model of the opened clamp to be constructed. Superposition of the relaxed β_1 onto domain 1 and the paired domain 3 at the interface that remains closed results in the appearance of a ≈15Å gap at the disrupted interface. This gap is large enough to accommodate single stranded DNA and we have proposed that inter-domain flexibility in addition to interaction with other subunits of the clamp loader might permit duplex DNA to enter/exit the clamp.

The 2.7/3.0 Å crystal structure of the clamp loader reveals a pentameric arrangement of subunits, with a stoichiometry δ:γ_3:δ (Figure 2) (2). The C-terminal domains of the subunits form a circular collar that supports an asymmetric arrangement of the N-terminal ATP binding domains of the γ motor and the structurally related domains of the δ' stator and the δ wrench. The nucleotide free clamp loader crystallizes with the β-interacting element of the δ wrench accessible to solvent and not buried within the assembly. This observation was unexpected since the clamp loader has no detectible interaction with the clamp in the absence of nucleotide. The 3 ATP binding sites on the γ motor subunits are located near clamp loader subunit interfaces. The structure crystallizes with only 2 of 3 sites available for ATP binding; the third site is blocked by the presence of structural elements from a neighboring subunit. We hypothesize that the structure in the crystal represents an intermediate in the clamp loading reaction. Nucleotide hydrolysis likely involves a conserved arginine residue from a neighboring subunit in a manner reminiscent to mechanisms proposed for other AAA+ ATPases (e.g. p97, NSF). The structure suggests a mechanism by which the g complex switches between a closed state, in which the β-interacting element of δ is hidden by δ, and an open form similar to the crystal structure, in which δ is free to bind to β.

Crystal structures of assemblies that are members of the (AAA+, ATPases associated with various cellular activities) family have emphasized the importance of subunit interfaces in the coupling of ATP binding/hydrolysis to biological function and inter-subunit communication. For example, in the NSF (6) (14) or P97 (15)assembly, the AAA+ domains are arranged as a hexamer with nucleotides bound near subunit inter-

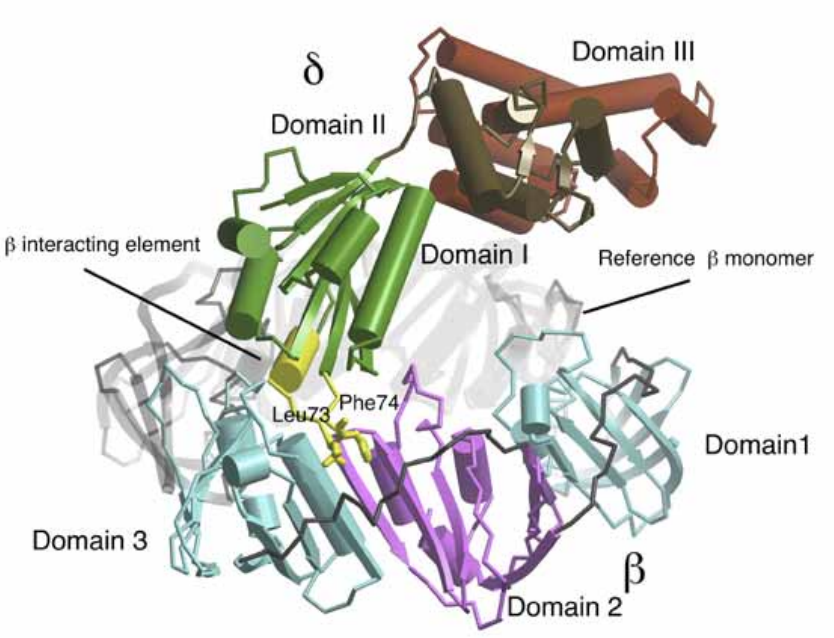

Figure 2. *Structure of the γ complex.*

faces. The majority of protein contacts to nucleotide come from the recA-like fold of one subunit, while inter-subunit communication and nucleotide hydrolysis are mediated, at least in part, by completion of the binding site through donation of a lys (NSF) or arg (p97) residue from an adjacent subunit. While the AAA+ proteins NSF and P97 crystallized as symmetric oligomers, the processivity clamp loader has been captured with the AAA+ modules deployed asymmetrically. Though a result of packing in the crystal lattice, this asymmetry may reflect one of the conformational states sampled by the clamp loader during the reaction. By analogy with other structures of AAA+ proteins, the ATP binding surfaces are aimed at neighboring subunits. In the structure of the bacterial clamp loader, only 2 of the 3 ATP binding sites are accessible for substrate binding; the third site, between γ1 and γ2 is blocked by invasion of residues (esp. val164) from an adjacent subunit. In the two ATP binding that are accessible to nucleotide, a conserved Arg residue from a neighboring subunit is located nearby ready to assist in hydrolysis. Use of an Arg residue from a neighboring subunit to facilitate ATP hydrolysis is reminiscent of the mechanism ('Arginine finger') utilized by the GTPase activating proteins (GAPs) to accelerate the hydrolysis of GTP by the Ras protein (1).

Surprisingly, the clamp loader crystallized with the β interaction element (BIE) exposed to solvent and not buried within the assembly as would have been expected for a nucleotide-free assembly. Attempts to build a model of the clamp-loader:clamp complex revealed that although the BIE is exposed to solvent, the clamp loader is insufficiently open to accommodate a dimeric clamp without steric clash. Only modest structural changes to the conformation seen in the crystal are required to accommodate the fully opened clamp.

We have proposed a clamp loading reaction scheme that incorporates the results of biochemical experiments and those from the structural analysis. In the absence of ATP, the clamp loader has no detectible interaction with the sliding clamp. Such a 'closed' model of the clamp loader can be easily generated by blocking the accessible ATP binding site on γ2 with the γ3 subunit, analogous to the γ1-γ2 interaction. We have proposed that preservation of the γ3-δ orientation would lead to burial of the beta interaction element within the δ' subunit. A tight δ–δ' interaction is consistent with biochemical studies. The observed rigidity of the δ' stator suggests that in the closed model of the clamp loader the ATP binding site on γ1 would remain accessible, while the other two ATP binding sites would be blocked. ATP would first bind to the open nucleotide binding site on γ1 at its interface with δ'. Nucleotide binding at the first site would lead to cooperative structural changes in the adjacent AAA+ modules of γ2 and γ3 that would unblock the second and third nucleotide binding sites. Occupancy of all the nucleotide binding sites would then expose the beta interaction element for contacts with the sliding clamp as seen in the structure of the β:δ assembly. Presence of primed-template, which has been properly positioned by the clamp loader within the opened clamp, would trigger hydrolysis of ATP. Hydrolysis of ATP would return the clamp loader to its 'closed' state and lead to release of clamp. The released clamp would close on DNA at the primer template junction. Experimental analysis of this proposed reaction scheme is currently underway.

References

(1) Ahmadian, M.R., et al., "Confirmation of the arginine-finger hypothesis for the GAP-stimulated GTP-hydrolysis reaction of Ras". Nat Struct Biol, 1997. **4**, 686-9.

(2) Jeruzalmi, D., M. O'Donnell, and J. Kuriyan, "Crystal structure of the processivity clamp loader gamma (gamma) complex of E. coli DNA polymerase III". Cell, 2001. **106**, 429-41.

(3) Jeruzalmi, D., et al., "Mechanism of processivity clamp opening by the delta subunit wrench of the clamp loader complex of E. coli DNA polymerase III". Cell, 2001. **106**, 417-28.

(4) Kelman, Z. and M.O. Donnell, "DNA Polymerase III Holoenzyme: Structure and Function of a Chromosomal Replicating Machine". Annual Reviews of Biochemistry, 1995. **64**, 171-200.

(5) Kuriyan, J. and M. O'Donnell, "Sliding clamps of DNA polymerases". J. Mol. Biol., 1993. **234**, 915-925.

(6) Lenzen, C.U., et al., "Crystal structure of the hexamerization domain of N-ethylmaleimide- sensitive fusion protein". Cell, 1998. **94**, 525-36.

(7) Neuwald, A.F., et al., "AAA+: A class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes". Genome Res, 1999. **9**, 27-43.

(8) O'Donnell, M., et al., "Homology in accessory proteins of replicative polymerases—E. coli to humans". Nucleic Acids Res, 1993. **21**, 1-3.

(9) Stewart, J., et al., "Mechanism of beta Clamp Opening by the delta Subunit of Escherichia coli DNA Polymerase III Holoenzyme". J Biol Chem, 2001. **276**, 19182-9.

(10) Stillman, B., "Smart machines at the DNA replication fork". Cell, 1994. **78**, 725-8.

(11) Stukenberg, P.T., P.S. Studwell-Vaughan, and M. O'Donnell, "Mechanism of the sliding beta-clamp of DNA polymerase III holoenzyme". J Biol Chem, 1991. **266**, 11328-34.

(12) Turner, J., et al., "The internal workings of a DNA polymerase clamp-loading machine". EMBO J, 1999. **18**, 771-83.

(13) Young, M.C., M.K. Reddy, and P.H. von Hippel, "Structure and function of the bacteriophage T4 DNA polymerase holoenzyme". Biochemistry, 1992. **31**, 8675-90.

(14) Yu, R.C., et al., "Structure of the ATP-dependent oligomerization domain of N-ethylmaleimide-sensitive factor complexed with ATP". Nat Struct Biol, 1998. **5**, 803-811.

(15) Zhang, X., et al., "Structure of the AAA ATPase p97". Mol Cell, 2000. **6**, 1473-84.